REMARKS

The above amendments to the above-captioned application along with the following remarks are being submitted as a full and complete response to the Official Action dated September 22, 2005.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

Status of the Claims

Claims 1-2 are under consideration in this application. Claims 1 and 2 are being amended, as set forth above, in order to more particularly define and distinctly claim Applicants' invention, while claims 3, 4 and 8-14 are being canceled without prejudice or disclaimer. Claims 5-7 were canceled in a previous response. Support for the claims may be found throughout the specification including, for example, on page 21, lines 5-18. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

Prior Art Rejections

In the Final Office Action, the Examiner maintained the rejection of claims 1–4 and 6-9 under 35 U.S.C. § 102(b) on the grounds of being anticipated by Nyren et al (WO 98/13523). The Examiner also again rejected claim 10 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of the article to Ishikawa et al (Human Immunology (1995) 42:315-318). Further, the Examiner maintained the rejection of claims 1 – 4, 8 – 9, 11 , 12 and 14 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of Nyren et al (WO 98/28440). Even more, the Examiner maintained the rejection of claims 10 and 13 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of Nyren et al (WO 98/28440), and further in view of Ishikawa et al. These rejections have been carefully considered, but are most respectfully traversed.

The present invention as now recited in claim 1 is directed to a method of analysis of DNA sequence, comprising the steps of: treating a deoxynucleotide solution containing deoxynucleotides for a complementary strand extension reaction by degrading, using pyrophosphatase, pyrophosphoric acid contained in the deoxynucleotide solution; removing or inactivating the pyrophosphatase in the deoxynucleotide solution after the pretreating step;

mixing the deoxynucleotide solution with reaction solution that contains a DNA primer, a target nucleic acid and a reagent for the extension reaction on the DNA primer, after the step of removing or inactivating; conducting the extension reaction on the DNA primer hybridized to the target nucleic acid, the extension reaction consisting of a plurality of one base extensions, wherein the deoxynucleotide solution is added to the reaction solution per each of said plurality of one base extensions; and detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step, wherein the deoxynucleotide solution does not contain the DNA primer, the nucleic target acid and the reagent.

As discussed in the previous response, Applicants will point out that the four dNTPs that are applicable to the present invention generate PPi (pyrophosphoric acid) by thermal degradation or the like and become the largest causes of signal noise and that, depending on the company from which a reagent was purchased, the manufacturing methods used, the lot and storage conditions, etc., the amounts of PPi contained as impurities in the four dNTPs or analogs thereof differ (page 27, lines 11-18). In order to reduce the cause of noise brought by too much PPi (pyrophosphoric acid) that is generated by thermal degradation of the nucleic acid substrate (dNTPs), the invention incorporates "treating a deoxynucleotide solution containing deoxynucleotides for a complementary strand extension reaction by degrading, using pyrophosphatase, pyrophosphoric acid contained in the deoxynucleotide solution; removing or inactivating the pyrophosphatase in the deoxynucleotide solution after the pretreating step; mixing the deoxynucleotide solution with reaction solution that contains a DNA primer, a target nucleic acid and a reagent for the extension reaction on the DNA primer, after the step of removing or inactivating; conducting the extension reaction on the DNA primer hybridized to the target nucleic acid, the extension reaction consisting of a plurality of one base extensions, wherein the deoxynucleotide solution is added to the reaction solution per each of said plurality of one base extensions; and detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step, wherein the deoxynucleotide solution does not contain the DNA primer, the nucleic target acid and the reagent, all as recited in claim 1. Here, pyrophosphatase breaks the phosphate bond which is exists not only in PPi, but also dNTP.

Again as noted previously, at the initial stage of treating using pyrophosphatase, the amount of dNTP is much more than the amount of PPi and the amount of degraded dNTP can be negligible. However, after the degradation of PPi, pyrophosphatase degrades only the dNTP. That is, pyrophosphatase will degrade too much dNTP, if it remains in the solution for

a long time. This results in the loss of dNTP and low sensitivity in the detection. To solve this problem, the invention includes the characteristics of removing or inactivating the pyrophosphatase in the deoxynucleotide solution after the step of treating. The above characteristic of the invention can reduce the cause of noise while avoiding excess dNTP degradation, and thereby allowing sensitive detection of the chemiluminescence reaction.

Applicants respectfully contend that none of the cited prior art references teaches or suggests the combination of steps or features as noted above, as recited for present the invention.

In contrast, regarding the rejection under 35 USC §102, Nyren et al. (PCT WO 98/13523) is only directed to removing ATP from reagent solution prior to addition to reaction mix (p.7, lines 18-20). In order to achieve this action, Nyren '523 discloses that the solution is contacted with immobilized enzyme that converts ATP into a product which is no longer a substrate for luciferase (p.7, lines 20-23). Nyren '523 does not disclose or suggest using pyrophosphatase for degrading PPi (pyrophosphoric acid) in a deoxynucleotide solution, as does the present invention. Therefore, Nyren '523 cannot, by itself, anticipate or render obvious each and every feature of the present invention as claimed.

Further, with respect to the rejection under 35 USC §103, Nyren '523 merely discloses that the immobilized enzyme may be removed prior to the chain extension/detection (p.7, lines 25-26). However, Applicants will point out that the target to be removed is apyrase degrading for ATP. Nyren '523 does not disclose or suggest at least "using pyrophosphatase for degrading PPi (pyrophosphoric acid) in a deoxynucleotide solution, as claimed for the invention. Thus, Nyren '523 cannot, by itself, render obvious any step to reduce the cause of noise with avoiding surplus dNTP degradation and realizes sensitive detection for chemiluminescence reaction.

The secondary reference of Nyren et al. (PCT WO98/28440) does not show or suggest any step of removing or inactivating the pyrophosphatase in the deoxynucleotide solution after the step of treating, such as that recited in the claims. Nyren '840 merely discloses that "any possible contamination of the reagents e.g. the NTP solutions, by PPi is undesirable and may readily be avoided by including a pyrophosphatase, preferably in low amounts, in the reagent solutions" (see p. 19, lines 2-9). However, as described above, pyrophosphatase breaks the phosphate bond not only in PPi, but also in dNTP. Thus, if the pyrophosphatase remains in the NTP solution even in low amounts, it will continually degrade the NTP, thus inevitably resulting in excessive degradation of NTP and low detection sensitivity. Further, to the extent

that Nyren '840 describes adding a pyrophosphatase in low amounts in the reagent solutions, or even allowing a pyrophosphatase to remain in low amounts, this reference falls far short of disclosing or even suggesting any step of removing or inactivating the pyrophosphatase in a deoxynucleotide solution after the step of treating. Therefore, Nyren '840 cannot and does not provide any disclosure or suggestion to make up for the deficiencies of Nyren '523 such that their combination can show or suggest at least the above features of the present invention as claimed, nor the advantages achieved through those features of the present invention.

With respect to the secondary reference of Ishikawa, again this reference merely describes the use of primers for detecting a single base difference between A2 alleles and other HLA-A alleles, having one extra mismatch at the second position from its 3'-end (See Abstract). Ishikawa fails to provide any teaching or suggestion that would make up for the deficiencies in Nyren '523 or in Nyren '840, as described above, such that their combination could render the features of the present invention obvious. In other words, even if these three references were combined, that combination would still fall short of embodying all the claimed features of the present invention. Thus, the present invention as claimed cannot be rendered obvious in view of Nyren '523, Nyren '840 and Ishikawa.

Conclusion

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art references upon which the rejections in the Office Action rely, Applicants respectfully contend that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of the above-captioned application, the Examiner is invited to contact the Applicants' undersigned representative at the address and telephone number indicated below.

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